The Use of Uterine Vascular Clamping in the Pregnant Rat to Modify the Embryotoxic Effects of Anticancer Drugs

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SUMMARY

The technic of isolating one horn of the pregnant rat uterus from the maternal circulation has been utilized to study the rapidity of action of cancer chemotherapeutic agents upon the developing embryo.

The eight- and nine-day-old rat embryo was the most suitable stage of gestation for studying the combination of uterine vascular clamping and the administration of anticancer drugs.

The four parameters that were utilized to evaluate the protective effect of uterine vascular clamping were the embryonic resorption rate, fetal growth retardation, incidence of congenital malformations, and fetal hematologic effects. The resorption rate was the most reliable parameter for measuring the protective effect of uterine vascular clamping. Although embryonic growth retardation was also a reliable parameter, the number of surviving embryos frequently was so small in one particular group that the weight data could not be utilized. Obtaining the incidence of malformations and the fetal blood counts was time consuming and yielded nondiscriminating results.

The combination of uterine vascular clamping plus the administration of nitrogen mustard, aminopterin, 5-fluorouracil, and 5-fluorodeoxyuridine demonstrated that the embryonic effect of the latter three drugs was ameliorated by isolating the pregnant uterus from the maternal circulation for 15 or 30 minutes. Surprisingly, the clamping technics did not protect against the lethal effects of nitrogen mustard. This was believed to be due to the persistence of clinical toxicity of this drug in the maternal rat.

The advantages and disadvantages of this technic are compared to other bioassay procedures. Since the interpretation of this bioassay procedure includes complex interrelationships between mother and embryo and many aspects of placental transfer, the practical usefulness of this bioassay procedure is limited to the determination of the biologic life span of cancer chemotherapeutic agents as they pertain to the embryo.

INTRODUCTION

The technic of isolating the uterus of the pregnant rat was first described in this laboratory in 1960 (4). The technic consists of applying special hemostatic forceps to the ovarian and cervical ends of one or both horns of the pregnant rat uterus, completely interrupting its blood supply. In this way one or both uterine horns and their embryonic contents can be isolated from the maternal circulation for periods up to three hours with some embryos still surviving (10, 11). There are several interesting consequences of this technic. Isolation of the pregnant uterus from the maternal circulation for one to three hours at seven, eight, nine, or ten days of gestation results in an increase in embryonic mortality, fetal growth retardation, and the production of congenital malformations. Thus, the effects of one to three hours of uterine clamping are quite similar to those seen with other teratogenic agents (11). On the other hand, if the pregnant uterus is isolated for less than one hour on certain days of gestation, minimal or no alterations are produced. Thus, it is possible to isolate the nine-day pregnant rat uterus for 30 minutes and have the term fetuses appear normal with regard to lethality, growth, and malformations (4, 10).

Since the mammalian embryo is frequently sensitive to cancer chemotherapeutic agents, it was felt that the uterine vascular clamping technic would provide one method for evaluating the rapidity of action of anticancer drugs on various aspects of embryonic development. A preliminary report of this study has been published (35).

This project had three main goals: (a) to determine how the uterine vascular clamping technic compares with other technics in determining the biologic effectiveness and rate of elimination or inactivation of various chemotherapeutic agents; (b) to determine which of the parameters measured, if any, are sensitive indicators of the protective effect of uterine vascular clamping (fetal death, fetal growth, fetal malformations, and hematologic status of the term fetus); (c) to determine on which day of gestation the protective effect is most likely to be manifested.

MATERIALS AND METHODS

Four hundred sixty-seven pregnant Wistar rats were utilized in this study. The pregnant rats were obtained from our own randomly inbred colony. Males and females were placed together overnight, and the following morning the females were examined for the presence of sperm in their vaginas. The inseminated females were considered to be 0 hours, 0 days pregnant at 9 a.m. on the morning that sperm were found. On a particular day of gestation, the pregnant rat was anesthetized...
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* Standard deviation.

with sodium pentobarbital, 30 mg/kg, and was placed in the supine position on an operating board. The abdomen was shaved and cleansed and a laparotomy performed. Both horns of the pregnant uterus were exteriorized, and the number and condition of the embryonic sites were recorded. One horn of the uterus was randomly chosen to be clamped. A special vascular clamp (4, 11) was placed across the ovarian vessels and the ovarian end of the pregnant uterus. A similar clamp was placed across the uterine vessels and the cervical end of the uterus. In the experimental groups, a particular anticancer drug was injected into the femoral vein as soon as the uterine horn was clamped and isolated from the maternal circulation. After either 15 or 30 minutes, the clamps were removed, the uterus replaced, and the abdomen closed with 3-0 silk sutures. The skin was closed with metal wound clips. The pregnant rat was sacrificed after 21 days of gestation. The fetuses were delivered by cesarean section, weighed, and examined. The fetal mortality was based on the number of implantation sites present at the time of the operative procedure and the number of fetuses alive at term. Control embryos were derived from litters which had one horn clamped but which had received no anticancer drug.

Four anticancer drugs were studied: aminopterin, nitrogen mustard, 5-fluorouracil, and 5-fluorodeoxyuridine. The dosage chosen was based on preliminary experiments that produced no less than a 30% embryonic mortality and no more than a 95% embryonic mortality in the unclamped embryos. The dosages utilized were aminopterin, 0.125 and 0.20 mg/kg; nitrogen mustard, 0.6 mg/kg; 5-fluorouracil, 50 mg/kg; and 5-fluorodeoxyuridine, 50 mg/kg. These drugs were administered to animals after eight (Table 1), nine (Table 2), ten, and seventeen days of pregnancy. In addition, aminopterin was administered to pregnant rats after 11, 12, and 13 days of pregnancy.

Besides the studies dealing with fetal mortality and fetal growth retardation, some hematologic investigations were undertaken. Rats pregnant seventeen days had one uterine horn clamped, were injected with one of the four anticancer drugs

### Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Maternal dose (mg/kg)</th>
<th>Clamping period (min)</th>
<th>Number of embryos</th>
<th>Number of term fetuses</th>
<th>Mortality at term (%)</th>
<th>Term fetal weight (gm)</th>
<th>Number of embryos</th>
<th>Number of term fetuses</th>
<th>Mortality at term (%)</th>
<th>Term fetal weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen mustard</td>
<td>0.6</td>
<td>30</td>
<td>57</td>
<td>40</td>
<td>29.8</td>
<td>4.33 ± 0.36*</td>
<td>53</td>
<td>29</td>
<td>45.6</td>
<td>3.99 ± 0.45</td>
</tr>
<tr>
<td>5-fluorouracil</td>
<td>50</td>
<td>15</td>
<td>47</td>
<td>1</td>
<td>97.8</td>
<td>4.12</td>
<td>41</td>
<td>12</td>
<td>70.8</td>
<td>5.20 ± 0.42</td>
</tr>
<tr>
<td>Aminopterin</td>
<td>0.125</td>
<td>30</td>
<td>88</td>
<td>4</td>
<td>95.6</td>
<td>3.96 ± 0.41</td>
<td>69</td>
<td>26</td>
<td>62.4</td>
<td>4.37 ± 0.75</td>
</tr>
<tr>
<td>5-fluorodeoxyuridine</td>
<td>0.2</td>
<td>30</td>
<td>38</td>
<td>0</td>
<td>100.0</td>
<td>3.32 ± 0.20</td>
<td>36</td>
<td>0</td>
<td>100.0</td>
<td>4.02 ± 0.70</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>30</td>
<td>73</td>
<td>8</td>
<td>89.1</td>
<td>4.79 ± 0.45</td>
<td>68</td>
<td>55</td>
<td>19.1</td>
<td>4.37 ± 0.65</td>
</tr>
<tr>
<td>Control</td>
<td>0.125</td>
<td>30</td>
<td>74</td>
<td>69</td>
<td>6.8</td>
<td>5.03 ± 0.43</td>
<td>75</td>
<td>12</td>
<td>16.0</td>
<td>4.59 ± 0.54</td>
</tr>
</tbody>
</table>

The modifying effect of uterine vascular clamping upon the lethal and growth-retarding characteristics of various chemotherapeutic agents to embryos nine days old.

* Standard deviation.

### Table 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>Maternal dose (mg/kg)</th>
<th>Clamping period (min)</th>
<th>Number of embryos</th>
<th>Number of term fetuses</th>
<th>Mortality at term (%)</th>
<th>Term fetal weight (gm)</th>
<th>Number of embryos</th>
<th>Number of term fetuses</th>
<th>Mortality at term (%)</th>
<th>Term fetal weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>30</td>
<td>51</td>
<td>46</td>
<td>9.8</td>
<td>4.54 ± 0.55*</td>
<td>33</td>
<td>28</td>
<td>15.0</td>
<td>4.57 ± 0.46</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>60</td>
<td>40</td>
<td>38</td>
<td>5.0</td>
<td>4.91 ± 0.36</td>
<td>29</td>
<td>21</td>
<td>28.0</td>
<td>4.57 ± 0.46</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>0.8</td>
<td>30</td>
<td>61</td>
<td>25</td>
<td>41.0</td>
<td>4.53 ± 0.26</td>
<td>53</td>
<td>27</td>
<td>49.1</td>
<td>4.32 ± 0.23</td>
</tr>
<tr>
<td>5-fluorouracil</td>
<td>50</td>
<td>15</td>
<td>47</td>
<td>1</td>
<td>97.8</td>
<td>3.89 ± 0.75</td>
<td>55</td>
<td>40</td>
<td>72.8</td>
<td>5.20 ± 0.42</td>
</tr>
<tr>
<td>5-fluorodeoxyuridine</td>
<td>50</td>
<td>30</td>
<td>36</td>
<td>3</td>
<td>91.8</td>
<td>3.89 ± 0.75</td>
<td>39</td>
<td>20</td>
<td>51.4</td>
<td>4.81 ± 0.39</td>
</tr>
<tr>
<td>Aminopterin</td>
<td>0.125</td>
<td>30</td>
<td>50</td>
<td>12</td>
<td>76.0</td>
<td>3.89 ± 0.75</td>
<td>35</td>
<td>27</td>
<td>22.8</td>
<td>4.44 ± 0.43</td>
</tr>
</tbody>
</table>

The modifying effect of uterine vascular clamping upon the lethal and growth-retarding characteristics of various chemotherapeutic agents to embryos nine days old.

* Standard deviation.
RESULTS

Eight-Day-Pregnant Rats. The results of uterine vascular clamping in 8-day-pregnant rats are summarized in Table 1. There are seven experimental groups in which an anticancer drug was administered in combination with uterine vascular clamping. The two control groups demonstrate that 15 minutes of vascular clamping neither increases mortality nor decreases the term fetal weight. Thirty minutes of uterine vascular clamping produces a reduction in fetal weight at the P < 0.05 level of significance, and the mortality on the clamped side is at the upper limit of normal for operated, unclamped embryos. Thus, neither 15 nor 30 minutes of clamping in the 8-day-pregnant rat drastically altered the growth and mortality of the fetuses at term. Previous studies have demonstrated that there is no significant increase in the malformation rate following this procedure (11). When the administration of nitrogen mustard (0.6 mg/kg) was combined with uterine vascular clamping, the unclamped embryos had a mortality of 19.8%, while the clamped embryos had a mortality of 45.6% (P < 0.02). The clamped embryos also had a significantly lower fetal weight (3.99 gm) when compared with the unclamped embryos (4.33 gm) (P < 0.01). Certainly, the uterine vascular clamping technic did not protect the embryos from the effects of nitrogen mustard. On the other hand, the lethal and growth-retarding effects of aminopterin, 5-fluorouracil, and 5-fluorodeoxyuridine were significantly reduced in those embryos clamped for 15 minutes (Table 1). In fact, comparisons for fetal weight and fetal mortality between the clamped and unclamped embryos exposed to all three drugs were significantly different at least at the P < 0.02 level of significance. In the groups receiving 50 mg/kg of 5-fluorouracil and 5-fluorodeoxyuridine, those embryos clamped for 30 minutes had a significantly lower mortality than did the embryos clamped for 15 minutes. The level of significance was P < 0.001 for 5-fluorouracil and P < 0.02 for 5-fluorodeoxyuridine. Thus, three of the four compounds tested in 8-day-old embryos had their growth-retarding and lethal effects ameliorated by isolating the embryos from the maternal circulation for 15 or 30 minutes.

Nine-Day Pregnant Rats. Similar results were obtained one day later in that uterine vascular clamping protected against the growth-retarding and lethal effects of aminopterin, 5-fluorouracil, and 5-fluorodeoxyuridine, but did not protect against the effects of nitrogen mustard (Table 2). With 5-fluorouracil, a 30-minute period of clamping afforded significantly more protection than 15 minutes of clamping (P < 0.05). Nine-day-old control embryos clamped for 30 minutes or less had the same mortality and growth rates as the unclamped control embryos (Table 2). Even after 60 minutes of clamping, the mortality of the clamped control embryos was only 28.0% (11).

<table>
<thead>
<tr>
<th>Table 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug and dose</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>0.6 mg/kg nitrogen mustard</td>
</tr>
<tr>
<td>50 mg/kg 5-fluorouracil</td>
</tr>
<tr>
<td>50 mg/kg 5-fluorodeoxyuridine</td>
</tr>
<tr>
<td>0.125 mg/kg of aminopterin</td>
</tr>
<tr>
<td>0.125 mg/kg of 5-fluorouracil</td>
</tr>
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</table>

The hematoxic response of the 17-day-pregnant rat and her fetuses to various chemotherapeutic agents, and the comparative response of fetuses which have been isolated from the maternal circulation by uterine vascular clamping during the 8th day of pregnancy is shown in Table 3.

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Aminopterin Plus Clamping in 8- to 13-Day-Old Embryos. This series of experiments was undertaken to determine whether there was an optimal stage of gestation for demonstrating a protective effect from the clamping procedure. Apparently 30 minutes of clamping becomes more detrimental as the gestational stage increases, as evidenced by an increase in mortality in the clamped embryos not exposed to the aminopterin. Furthermore, the same dose of aminopterin becomes less effective as the embryo matures. A dose of 0.125 mg/kg of aminopterin results in 95.6%, 61.4%, and 35.4% resorptions when administered to 8-, 11-, and 13-day-old embryos respectively. Thus, the older embryos become more resistant to the lethal effects of aminopterin and less resistant to the lethal effects of vascular clamping. This combination would lead one to predict that the protective effect of uterine vascular clamping observed in the eight- and nine-day-old embryos would be markedly reduced or absent in older embryos. Actually the 12- and 13-day-old embryos had higher mortalities on the clamped side than on the side which was exposed to aminopterin. It appears that in the older embryos, the detrimental effects of uterine vascular clamping negated any benefit obtained from isolating the pregnant uterine horn for thirty minutes.

Congenital Malformations. All four anticancer drugs utilized in this study have been reported to produce congenital malformations (1, 9, 13, 15, 16, 23-25, 33). Because of the brief periods of clamping, there was no increase in malformations in any of the groups subjected to vascular clamping alone. The high fetal mortality in the treated embryos that were not clamped precluded any valid evaluation of the incidence of malformations because of the small number of term fetuses in these groups.

Hematologic Response of Mother and Embryos to Anticancer Drugs. A hemoglobin, white blood cell count, platelet count, and red blood cell count were performed at term on the maternal rats and fetuses. Ten mothers that received aminopterin and 5-fluorouracil after either eight or nine days of pregnancy had normal hematologic findings at term. Thirty-one fetuses from these mothers were also examined hematologically, and the surviving clamped and unclamped fetuses had similar blood counts. These counts were also the same in control term fetuses. Thus, neither the clamping nor the drugs administered early in gestation affected the hematologic findings in term fetuses.

Animals that were administered the anticancer drugs after 17 days of pregnancy were also evaluated hematologically (Table 3). The hemoglobin and white blood count, when measured four days later in the maternal rats, were lower in all four groups that were administered the experimental drugs. The red blood cell count was depressed in all but the nitrogen mustard group. The platelets did not seem affected in any of the experimental groups. There was no question that nitrogen mustard, aminopterin, 5-fluorouracil, 5-fluorodeoxyuridine produced a significant hematologic effect in the maternal rat four days after the administration of any of these drugs.

On the other hand, the hematologic picture in the clamped and unclamped fetuses was quite normal. There were no significant differences between the hemoglobin, white blood count, and red blood count in the clamped and unclamped embryos at the $P < 0.01$ level of significance. Although the platelet counts in the nitrogen mustard group and the 5-fluorouracil group were lower in the unclamped fetuses, this difference was not significant at the $P < 0.05$ level of significance. As a group, the term fetuses demonstrated almost no hematologic effect when examined four days after the mother had received one of the four cancer chemotherapeutic agents, although the maternal blood picture had been markedly affected (Table 3).

DISCUSSION

The biologic life span of various anticancer drugs can be studied by many methods. Drugs labeled with radioisotopes provide a very accurate method of determining their rates of disappearance from the circulation and their quantitative depositions in various tissues. More tedious chemical determinations or microbiologic assays have also been utilized (6, 20, 21). Determination of the rate of disappearance and deposition of an anticancer drug is only one clue to its biologic effects and toxicity. There are biologic methods of drug evaluation which are similar to the technic described in this paper. These other methods depend upon the marked sensitivity of the hematopoietic system and the intestinal epithelium to anticancer drugs. Karnofsky et al. (15) clamped off the circulation to portions of the intestine and bone marrow for various periods of time and were able to demonstrate histologically that short periods of clamping protected against the antiproliferative effects of nitrogen mustard. Using this technic, Ulfohn et al. (33) showed that it was possible to protect completely tissues with as little as 15 seconds of clamping following the administration of nitrogen mustard. These results differed markedly from our results with nitrogen mustards in which no protection was noted with 30 minutes of clamping. The reason for this difference is not apparent. Our dosage schedule (0.6 mg/kg) was significantly lower than theirs (2 mg/kg). Also, nitrogen mustard appeared to be the only drug used by us that caused significant maternal morbidity and mortality. This discrepancy is an important drawback to the vascular clamping technic if one wishes to evaluate the biologic life span of anticancer drugs. It appears that the simpler technics of protecting hematopoietic tissue or intestine are more closely akin to the clinical situation that exists in isolated, local arterial perfusions for tumor therapy (7, 8, 12, 14, 18, 21, 22, 26, 31). Therefore, the complexity of the maternal-placental-fetal interaction may inordinately complicate the ability to interpret pharmacologic effects. Apparently, in our experiments the systemic toxicity of nitrogen mustard in the mother was able to affect the embryos long after the acute anticancer effect of nitrogen mustards was gone. Therefore, it would appear that the uterine vascular clamping technic has limited application as a general test for the efficacy of anticancer drugs. On the other hand, this technic should be useful for specifically studying the pharmacologic effects of the drug on the embryo, fetus, and placenta.

For those interested in utilizing the uterine vascular clamping technic for studying the rapidity with which pharmacologic agents cross the placenta and affect the fetus, the following information is pertinent. Of the parameters measured (embryonic mortality, weight, malformation, and hematopoiesis), embryonic mortality was the most reliable and repro-
ducible. Furthermore, the 8- and 9-day-old rat embryos were the best stages for combining uterine vascular clamping and the administration of cancer chemotherapeutic agents for the purpose of determining how rapidly their embryotoxicity diminished.

In older embryos the clamping procedure alone produces an increase in fetal death (11). Therefore, the evaluation of drugs late in gestation is not feasible. This is clearly shown by the fact that vascular clamping protected against the lethal effects of aminopterin early in gestation, but later in gestation the detrimental effects of the clamping procedure predominated.

The hematologic data of term fetus exposed to anticancer drugs late in gestation (17-day-old fetuses) were most interesting (Table 3). Neither the control nor clamped 17-day-old fetuses exhibited any hematologic depression four days later at term, despite the fact that all the mothers exhibited significant hematologic depression. Thus, either the drugs did not reach the fetus or the hematopoietic tissue of the fetus recovers at a more rapid rate. Therefore, although fetal hematologic evaluation following uterine vascular clamping proved to be useless, the unexpected response of the fetal hematopoietic tissue warrants further investigation.

REFERENCES

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